Rapid publication

Immunologic changes in children with egg allergy ingesting extensively heated egg

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Background: Prior studies have suggested that heated egg might be tolerated by some children with egg allergy.
Objective: We sought to confirm tolerance of heated egg in a subset of children with egg allergy, to evaluate clinical and immunologic predictors of heated egg tolerance, to characterize immunologic changes associated with continued ingestion of heated egg, and to determine whether a diet incorporating heated egg is well tolerated.
Methods: Subjects with documented IgE-mediated egg allergy underwent physician-supervised oral food challenges to extensively heated egg (in the form of a muffin and a waffle), with tolerant subjects also undergoing regular egg challenges (in a form of scrambled egg or French toast). Heated egg–tolerant subjects incorporated heated egg into their diets. Skin prick test wheal diameters and egg white, ovalbumin, and ovomucoid IgE levels, as well as ovalbumin and ovomucoid IgG4 levels, were measured at baseline for all subjects and at 3, 6, and 12 months for those tolerant of heated egg.
Results: Sixty-four of 117 subjects tolerated heated egg, 23 tolerated regular egg, and 27 reacted to heated egg. Heated egg–reactive subjects had larger skin test wheals and greater egg white–specific, ovalbumin-specific, and ovomucoid-specific IgE levels compared with heated egg– and egg-tolerant subjects. Continued ingestion of heated egg was associated with decreased skin test wheal diameters and ovalbumin-specific IgE levels and increased ovalbumin-specific and ovomucoid-specific IgG4 levels.
Conclusions: The majority of subjects with egg allergy were tolerant of heated egg. Continued ingestion of heated egg was well tolerated and associated with immunologic changes that paralleled the changes observed with the development of clinical tolerance to regular egg. (J Allergy Clin Immunol 2008;122:977-83.)

Key words: *Egg allergy, hen's egg allergy, baked egg, heated egg, food allergy, intestinal permeability, oral food challenge*

Egg white is one of the leading causes of IgE-mediated food allergy in childhood, affecting approximately 1.6% of children[1,2]; the prevalence is considerably higher in children with atopic dermatitis or other food allergies.[3,4] Previously, it had been thought that the majority of allergic children become egg tolerant by school age.[5] However, a recent study has suggested that egg allergy persists well into the adolescent years for many children with egg allergy.[6] Clinical and laboratory factors that have been associated with the development of tolerance to egg include milder symptoms on egg ingestion, smaller skin test size, earlier age at diagnosis, specific egg IgE antibody levels, and rate of change of egg IgE antibody levels.[6] Persistence of egg allergy was associated with egg white IgE antibody levels of greater than 50 kUA/L[6] and with the presence of IgE antibodies directed at linearized ovomucoid.[7,8]

Ovomucoid is considered to be the dominant allergen in hen's egg white.[7,9-11] Although ovalbumin is the most abundant protein found in egg white, it is sensitive to thermal denaturation, with a resultant decrease in allergenicity.[12] In contrast, ovomucoid is heat resistant and remains soluble after extensive heating. Deutsch and Morton[13] found that purified ovomucoid heated for 1 hour at 100°C retained its antibody-binding activity. Cooke and Sampson[7] evaluated IgE binding to linearized versus native ovomucoid and reported that pooled sera from patients with egg allergy showed no significant differences in native ovomucoid binding, although sera from several patients with transient egg allergy showed a marked reduction in IgE binding to linearized ovomucoid. Jarvinen et al[8] found that IgE antibodies of children with persistent egg allergy recognized more linear ovomucoid epitopes than those of children with transient egg allergy, suggesting that different phenotypes exist among children with egg allergy. These studies suggested that ovomucoid conformational epitopes might be important in some patients with egg allergy.

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Abbreviations used
LacMan: Lactulose/mannitol ratio
SPT: Skin prick test

Urisu et al[14] reported that 21 of 38 subjects with egg allergy were tolerant of heated egg white food challenge. Of the 17 subjects who reacted to heated egg white, 16 showed tolerance on rechallenge to ovomucoid-depleted heated egg white. Another brief report indicated that 73% of children more than 5 years of age with a history of clinical reactivity to egg and a positive skin prick test (SPT) response tolerated egg baked into a cake during a physician-supervised oral food challenge.[15] An additional case report described an adult who experienced anaphylaxis to raw egg after an asymptomatic challenge to heated egg.[16] Collectively, these data suggest that extensive heating diminishes the allergenicity of egg white proteins and that subjects with egg allergy differ in their ability to mount IgE antibody responses against heated (denatured) egg white proteins.

We sought to determine whether a diet incorporating extensively heated hen's egg is tolerated by children with egg allergy and to characterize immunologic changes associated with introduction of heated egg into the diet. Additionally, we sought to identify clinical and immunologic parameters that predict tolerance of heated egg in children with egg allergy.

METHODS

Participants

Subjects with documented IgE-mediated egg allergy were recruited from the pediatric allergy clinics at the Mount Sinai Medical Center, New York. The study was approved by the Mount Sinai Institutional Review Board, and informed consent was obtained before enrollment.

Inclusion criteria

Subjects were eligible for enrollment based on the following criteria: age between 0.5 and 25 years; a positive SPT response to egg white, detectable serum egg white–specific IgE, or both and a recent history (within the past 6 months) of a type I hypersensitivity reaction to egg or a positive physician-supervised oral food challenge result to egg; or a serum egg-specific IgE level of greater than 7 kUA/L in children older than 2 years of age or greater than 2 kUA/L in children less than 2 years of age.

Exclusion criteria

Subjects were excluded from the study if they had a negative SPT response and undetectable serum egg white-specific IgE, had a recent reaction to extensively heated egg, had allergic eosinophilic esophagitis, had unstable asthma, used antihistamines more than 1 time within 3 to 7 days of challenge, or were pregnant.

Design

Tolerance to heated egg was determined by means of an oral food challenge (Fig 1). Subjects tolerant to heated egg were challenged with regular egg. Heated egg–reactive subjects were instructed to strictly avoid egg. Heated egg–tolerant subjects were instructed to incorporate heated egg products into their diets and were followed with monthly telephone calls to monitor adverse symptoms and adherence to diet, as well as follow-up visits at 3, 6, and 12 months. Subjects were advised to consume 1 to 3 servings of heated egg per day in the form of store-bought baked products with egg listed as the third or higher ingredient or homemade goods baked with 1 egg per 1 cup of flour or 1 to 2 eggs per batch of a recipe prepared according to temperature and time specifications for muffins and waffles administered during the study challenges.

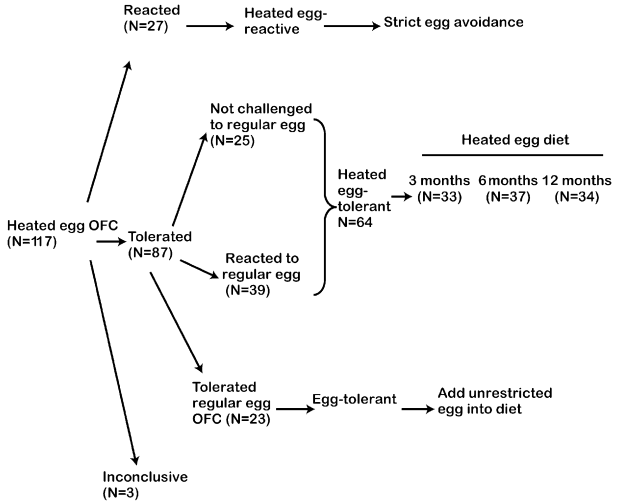

FIG 1. Study design and enrollment. *OFC,* Oral food challenge.

They were advised to strictly avoid Caesar salad dressing; custard; hard-boiled, scrambled, or poached eggs; French toast; frosting or icing; ice cream; mayonnaise; meringue; and quiche. Egg-tolerant subjects were instructed to incorporate all forms of egg into their diets.

Immunologic evaluation

SPTs were performed as previously described.[17] A serum sample was collected to measure egg white –, ovomucoid-, and ovalbumin-specific IgE and ovalbumin- and ovomucoid-specific IgG4 by using the UniCAP (Phadia, Portage, Mich) at each visit.

Oral food challenges to heated egg were performed openly under physician supervision in the Mount Sinai General Clinical Research Center. During each oral food challenge, a muffin and a waffle that each contained one third of an egg (approximately 2.2 g of egg protein) were ingested. The muffin was baked at 350°F for 30 minutes in an oven, and the waffle (<0.625 inches thick to ensure thorough heating) was cooked in a waffle maker at approximately 500°F for 3 minutes. Each food was administered in 4 equal portions over 1 hour. The muffin was served first; if no symptoms were observed, 2 hours later, the waffle was served. Participants who tolerated both the muffin and waffle challenges (heated egg tolerant) were challenged with regular egg if their test results were less than the 95% positive predictive values for an allergic reaction: an egg white–specific IgE level of greater than 7 kUA/L in children older than 2 years of age or greater than 2 kUA/L in children less than 2 years of age and an SPT wheal diameter of greater than 8 mm.[18-22] Increasing doses of scrambled egg or French toast were administered over 1 hour (total dose, 1 egg or 6.5 g of egg protein), as per routine protocol.[23-25] Subjects were monitored throughout and for 2 to 4 hours after the completion of the final challenge. Challenges were discontinued at the first objective sign of reaction, and treatment was initiated immediately.[23,24]

Anthropometric measurements

Anthropometric parameters were followed to monitor effects of heated egg ingestion on growth. Weight, height, and body mass index percentiles for age and *z* scores were calculated with Nutchildren (Epi Info 3.4.1; Centers for Disease Control and Prevention, Atlanta, Ga) for each study visit.

Intestinal permeability

To monitor for subclinical gastrointestinal hypersensitivity responses caused by the introduction of heated egg,[26] intestinal permeability was assessed at baseline for all toilet-trained subjects and at the 3- and 12-month follow-up visits for subjects ingesting the heated egg diet by means of a

measurement of urinary clearance of nonmetabolized sugars.[27] A lactulose/mannitol ratio (LacMan) of greater than 0.025 was considered abnormal based on the internal laboratory reference (Dr Jon Meddings, University of Alberta, Edmonton, Canada).

Statistics

ANOVA or ANOVA on ranks tests were used for determining statistical significance ($P < .05$) between continuous variables; a paired t test or Wilcoxon signed-rank test was used when comparing different time points with SigmaStat 3.5 (SYSTAT Software Inc, San Jose, Calif). Dichotomous variables were analyzed by using the χ^2 test. Predictive probabilities of the heated egg challenge outcome were calculated with logistic regression, as previously published.[19,20]

RESULTS

Baseline clinical characteristics

One hundred seventeen subjects with a mean age of 6.9 years (range, 1.6–18.6 years) were enrolled in the study from June 2004 to September 2007. Twenty seven subjects were heated egg reactive, 64 were heated egg tolerant, and 23 were egg tolerant. Three subjects had inconclusive heated egg challenges (because of refusal to eat the entire challenge food) and were excluded from the final analysis (Fig 1). Thus 70% (64/91) of children with egg allergy were tolerant to heated egg. Baseline clinical characteristics of the study subjects are outlined in Table E1 (available in this article's Online Repository at www.jacionline.org). Except for shorter duration of exclusive breast-feeding and higher lifetime peak egg white–specific IgE antibody levels, there were no significant differences between the study groups. We found no significant difference in the rate of tolerance to heated egg between younger (<5 years) and older (>5 years) children.

Oral food challenges

Allergic symptoms (Table I) ranged from mild to severe, although no subject had life-threatening anaphylaxis (hypotension, severe airway obstruction, and cardiac arrest). Five subjects reactive to heated egg received epinephrine. There were no significant clinical and immunologic differences between subjects reactive to heated egg who received and those who did not receive epinephrine. The median eliciting dose of heated egg protein was 2.0 g (range, 0.1-2.2 g).

Nine of 39 subjects reactive to regular egg (Fig 1 and Table I) received epinephrine. The median eliciting dose was 1.5 g (range, 0.01-6.5 g); 75% of subjects reacted at a dose lower than 2.2 g (equivalent to the amount served during heated egg challenge). Subjects treated with epinephrine during the regular egg challenge had higher median egg white– and ovomucoid-specific IgE levels (2.5 vs 0.7 kUA/L [$P = .046$] and 1.8 vs 0.5 kUA/L [$P = .004$], respectively) and higher median ovomucoid- and ovalbumin-specific IgE/IgG4 levels compared with those of subjects not treated with epinephrine (23.5 vs 1.5 kUA/L [$P = .019$] and 6.6 vs 2.4 kUA/L [$P = .037$], respectively). We found no significant correlation between egg white and ovomucoid-specific IgE antibody levels, SPT wheal sizes, and LacMan values and reaction severity scored by using a 1- to 5-point grading system.[28]

Twenty-five heated egg–tolerant subjects were not challenged to regular egg because of egg white–specific IgE (9 subjects) and SPT (5 subjects) values greater than the highly predictive levels of reactivity[19-23] or recent (within previous 6 months) convincing clinical reaction to regular egg (7 subjects). Four subjects refused the regular egg challenge.

TABLE I. Challenge data clinical reactions

Parameter*	Extensively heated egg OFC (n = 27)	Scrambled egg/French toast (n = 39)
Symptoms		
Cutaneous	11	24
UA	19	27
LA	4	4
GI	11	18
CV	0	0
Treatment		
Epinephrine + other medications	5	9
Diphenhydramine only	14	23
Diphenhydramine + other medications (except epinephrine)	4	4
No treatment†	4	3
Eliciting dose (g) of egg protein, median (range)	2.0 (0.1-2.1)	1.5 (0.01-6)

OFC, Oral food challenge; *Cutaneous*, atopic dermatitis, urticaria, angioedema, rash, and pruritus; *UA*, upper airway (rhinorrhea, nasal congestion, sneezing, and oral pruritus); *LA*, lower airway (wheezing); *GI*, gastrointestinal (nausea, vomiting, diarrhea, and abdominal pain); *CV*, hypotension.
Numbers in each column represent the number of patients with specific symptoms on oral food challenge. Numbers in parentheses represent ranges.
*No statistical differences were observed between groups for any parameter.
†Subjects not treated with medications had transient allergic symptoms, including abdominal pain, a single episode of emesis, scattered urticaria, and oral pruritus.

Probability curves

Probability curves were generated for reactivity to heated egg–for egg-specific IgE antibody levels and egg white–induced SPT wheal diameters (Fig 2). Ovomucoid-specific IgE levels showed the greatest predictive value; a level of 50 kUA/L was more than 90% predictive, whereas an undetectable level of ovomucoid-specific IgE still carried a 10% chance of heated egg reactivity. Although an egg white–induced SPT wheal diameter of 15 mm was only 60% predictive, a negative egg white–induced SPT response indicated less than a 5% chance of heated egg reactivity.

Baseline immunologic parameters

For more information on baseline immunologic parameters, see Table II. Subjects reacting to heated egg had significantly larger egg white–induced SPT wheal diameters and greater egg white–, ovalbumin-, and ovomucoid-specific IgE levels compared with those of subjects tolerant to heated egg and unheated egg. Ovalbumin- and ovomucoid-specific IgG4 levels did not differ significantly. Ovalbumin-specific and ovomucoid-specific IgE/IgG4 ratios were higher in heated egg–reactive subjects, reflecting the higher specific IgE levels (Table II).

Immunologic parameters in the subjects ingesting heated egg

For more information on immunologic parameters in the subjects ingesting heated egg, see Table III. Egg white–induced SPT wheal diameters decreased significantly, and ovalbumin- and ovomucoid-specific IgG4 levels increased from baseline at 3, 6, and 12 months but did not change significantly beyond 3 months (Table III). Ovalbumin-specific IgE levels were decreased at 12 months compared with baseline values, whereas egg white– and ovomucoid-specific IgE levels did not differ from baseline at any of the

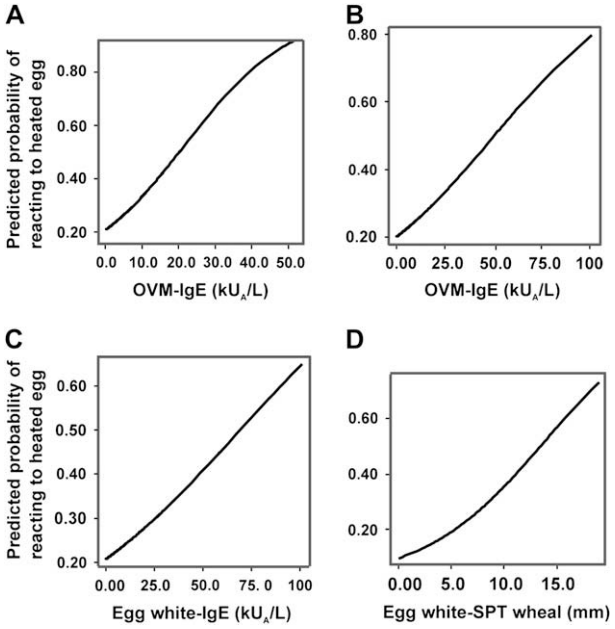

FIG 2. Predicted probabilities of the heated egg challenge (oral food challenge) outcome and serum egg white–specific IgE concentrations and SPT responses. Logistic regression was used to calculate the probability of reacting during heated egg oral food challenge in regard to serum ovomucoid *(OVM)*–specific IgE **(A)**, serum ovalbumin *(OVA)*–specific IgE **(B)**, serum egg white–specific IgE **(C)**, and egg white–induced SPT wheal size **(D)**.

follow-up intervals. Ovalbumin- and ovomucoid-specific IgE/IgG4 ratios were decreased from baseline values at 3, 6, and 12 months.

Anthropometrics

Anthropometric parameters did not differ between the study groups at baseline (data not shown). In the subjects ingesting the heated egg diet, there were no significant differences in weight, height, and body mass index percentiles for age and *z* scores between baseline and any of the follow-up times (Table IV).

Intestinal permeability

Intestinal permeability data were collected at baseline for all toilet-trained subjects and did not differ significantly among the 3 groups (not shown). LacMan values decreased compared with baseline values in the subjects ingesting heated egg at the 3-month visit (0.0255 vs 0.0203, $P = .019$) but were not significantly decreased at the 12-month follow-up (0.0240 vs 0.0215, $P = .9$). LacMan values did not correlate with tolerance to heated and unheated egg or with the severity of reactions during the oral food challenges to heated and unheated egg.

Tolerability of a heated egg diet

None of the subjects ingesting heated egg had documented worsening of an underlying allergic condition, such as atopic dermatitis, rhinitis, or asthma. There were no reported acute allergic reactions to heated egg at home.

Withdrawals

Eighteen subjects withdrew from the study after tolerating the initial heated egg challenge, and all were contacted by telephone. Sixteen subjects continued to ingest heated egg on a regular basis, and 1 incorporated scrambled eggs and French toast. All 16 subjects cited inconvenience as a reason for withdrawal. Of the remaining 2 subjects, 1 discontinued ingestion after 6 months because of parental perception of worsening of atopic dermatitis, although he was seen at 2 prior follow-up visits without an objective increase in atopic dermatitis severity. The other child did not like food products containing heated egg. These subjects declined further follow-up within the study.

DISCUSSION

We found that the majority (70%) of children with egg allergy tolerated extensively heated egg baked with wheat matrix, confirming prior observations that heating significantly diminishes the allergenicity of hen's egg white.[7,14,15] We report for the first time that incorporating extensively heated egg products into the daily diet is well tolerated, without any adverse effects on underlying allergic diseases, intestinal permeability, or normal growth. Furthermore, we report that regular ingestion of heated egg is associated with decreasing SPT wheal diameters to egg white and increasing ovalbumin-specific and ovomucoid-specific IgG4 antibody levels. The immunologic changes occur predominantly during the first 3 months after introduction of the heated egg and are maintained at later time points.

The current standard of care for children with egg allergy is strict dietary avoidance of egg-containing products.[29] However, the results of our study demonstrate that ingestion of extensively heated egg products is well tolerated and safe, suggesting that strict dietary avoidance of heated egg might not be necessary for the majority of patients with egg allergy.

We attempted to identify clinical and immunologic predictors of tolerance to heated egg. None of the clinical parameters achieved high predictive value, including a history of egg-induced anaphylaxis. Heated egg–tolerant subjects had lower lifetime peak egg white–specific IgE levels, smaller SPT wheal sizes, and lower egg white–, ovalbumin-, and ovomucoid-specific IgE levels than heated egg–reactive subjects at baseline. However, none of these parameters achieved sufficiently high predictive values, and thus an oral food challenge remains the only conclusive test to determine tolerance of heated egg.

The majority of studies have identified ovomucoid to be most important clinically, presumably because of its ability to maintain allergenicity despite extensive heating, acid treatment, or both. Our study confirms the importance of ovomucoid because heated egg–reactive subjects had higher ovomucoid-specific IgE levels compared with heated egg–tolerant and egg-tolerant subjects. Because ovomucoid is largely heat resistant, it follows that subjects with egg allergy who are tolerant of heated egg should have little clinically significant ovomucoid-specific IgE. The probability curves generated for heated egg reactivity provide further support because undetectable levels of ovomucoid-specific IgE are associated with less than a 10% chance of reactivity to heated egg. However, somewhat unexpected was the finding that only extremely increased (>50 kUA/L) levels of ovomucoid-specific IgE were highly predictive of heated egg reactivity. One explanation for this finding might be the so-called matrix effect.[30,31]

A recent study by Kato et al[32] demonstrated a marked decrease in the solubility of ovomucoid when egg white was mixed with wheat flour and wheat gluten and then heated at 180°C for 10 minutes, mimicking the process of bread making. Immunoblotting suggested that ovomucoid polymerizes and forms high-molecular-weight complexes, with gluten leading to aggregation and

TABLE II. Baseline immunologic parameters

	Heated egg reactive	Heated egg tolerant	Egg tolerant	*P* value
SPT wheal diameter (mm)	8 (6.3-9)	6 (5-8)	6 (5-6.8)	.019
EW-specific IgE6 mo before heated egg OFC (kUA/L)	5.7 (2.1-11.4)	1.6 (0.8-5.8)	1.1 (0.7-3.3)	.001
Egg-specific IgE (kUA/L)	5.1 (1.9-11.1)	1.3 (0.6-4.3)	1.0 (0.7-2.5)	.001
OVA-specific IgE (kUA/L)	7.0 (2.9-14.2)	1.6 (0.7-4.7)	1.0 (0.7-1.4)	<.001
OVM-specific IgE (kUA/L)	3.3 (0.7-13.7)	1.0 (0-3.0)	0.5 (0-1.1)	.019
OVA-specific IgG4 (μg/L)	0.2 (0-0.6)	0.4 (0-2)	0.1 (0-1.2)	Not significant
OVM-specific IgG4 (μg/L)	0.1 (0.01-0.2)	0.1 (0-0.4)	0.03 (0-0.12)	Not significant
OVA-specific IgE/IgG4 ratio	43.2 (19.1-130.9)	5.7 (1.7-81)	5.7 (0.4-76)	.011
OVM-specific IgE/IgG4 ratio	46.2 (6.7-91.3)	7.4 (2-14.5)	3.1 (0-19.8)	.012

EW, Egg white; *OFC*, oral food challenge; *OVA*, ovalbumin; *OVM*, ovomucoid.
Comparisons were made with ANOVA. All data reported represent median values with 25% to 75% interquartile ranges shown in parentheses.

TABLE III. Follow-up immunologic parameters in children ingesting extensively heated egg

	Baseline	3 mo	*P* value	6 mo*	12 mo*
SPT (mm)	6 (5-8)	5 (3.4-7)	<.001	5 (3-7)	5 (3.5-6)
EW-specific IgE (kUA/L)	1.3 (0.6-4.3)	1.7 (0.6-5.2)	NS	1.7 (0.7-4.7)	1.3 (0.3-3.2)
OVA-specific IgE (kUA/L)	1.6 (0.7-4.7)	1.4 (0.5-4.9)	NS	1.6 (0.6-4.5)	1.1 (0.5-3.2)
OVM-specific IgE (kUA/L)	1.0 (0-3)	0.8 (0-3.3)	NS	1.2 (0.3-2.8)	1.0 (0.3-2.3)
OVA-specific IgG4 (μg/L)	0.4 (0-2)	4.6 (0.6-12.5)	<.001	3.1 (0.5-7.6)	3.1 (0.9-10)
OVM-specific IgG4 (μg/L)	0.1 (0-0.4)	0.6 (0.1-1.4)	<.001	0.5 (0.1-1.5)	0.6 (0.1-1.7)
OVA-specific IgE/IgG4 ratio	5.7 (1.7-81)	0.6 (0.1-2.9)	<.001	0.7 (0.1-2.9)	0.4 (0.03-0.5)
OVM-specific IgE/IgG4 ratio	7.4 (2-14.5)	1.1 (0.07- 3.3)	<.001	1.4 (0-6.7)	0.9 (0.2-4.2)

EW, Egg white; *NS*, not statistically significant ($P > .05$); *OVA*, ovalbumin; *OVM*, ovomucoid.
All numbers represent median values with 25% to 75% interquartile ranges provided in parentheses.
*No significant changes were noted in any of the parameters between the 3-, 6-, and 12-month follow-up intervals.

insolubilization of ovomucoid. This decrease in the solubility of ovomucoid could explain why such high levels of ovomucoid-specific IgE are required to predict reactivity to heated egg in baked products.

The severity of reactions to unheated egg in the heated egg–tolerant subjects was in contrast with a recent study from our group examining tolerance to heated milk.[33] No subject tolerant to heated milk received epinephrine during the unheated milk challenge, suggesting that tolerance to heated milk is a predictor of a milder allergic phenotype. In the present study 18.5% of heated egg–reactive and 23% of regular egg–reactive subjects had anaphylaxis (none severe) and were treated with epinephrine, indicating that tolerance to heated egg is not predictive of milder clinical reactions to regular egg. This suggests that in the case of hen's egg white, interactions with the food matrix at high temperatures are important for decreasing allergenicity.

Our observation emphasizes that individual food behavior under heating conditions and interactions with the food matrix are highly variable and must be evaluated separately for each food. In fact, thermal denaturation was investigated for a number of food allergens.[30] Some food proteins are particularly heat labile (eg, proteins cross-reactive with tree pollen), but the majority of food allergens are relatively heat resistant[1]; furthermore, some food proteins are rendered more allergenic by thermal processing.[34-36]

There are several limitations inherent to the study design, which was driven by the clinical objectives. We chose to investigate extensively heated (muffin and waffle) and regular (scrambled egg or French toast) egg forms and the amounts that are typically ingested in the Western diet to make the results applicable to the daily life of children with egg allergy. Although the quantity of heated egg was less than the amount of regular egg administered during an oral food challenge, it was equivalent to the typical quantities found in the home-baked and commercial food products. Furthermore, 75% of children reacted to a dose of regular egg that was less than or equal to the amount of heated egg. Because of the practical constraints, we did not perform food challenges to higher doses of heated egg, to heated egg that was not baked with wheat matrix, and to raw egg white because those foods would be very unlikely to be encountered by children in their diet. It is possible that we would have observed different correlations between serum specific egg white–, ovomucoid-, and ovalbumin-specific IgE antibody levels and the tolerance of heated egg, as well as the severity of the reactions, most likely because of the confounding effect of the food matrix.

For ethical reasons, not all subjects tolerant to heated egg underwent a regular egg challenge because they were more than 95% likely to react as predicted by egg white–specific IgE levels and large egg-induced SPT wheal diameters or recent convincing clinical reactions to egg. In our follow-up study we plan to decrease the risk of a reaction by performing multistep challenges in all subjects to increasingly less-heated forms of egg, including regular egg. It should be noted that subjects enrolled in the current study were carefully preselected to minimize the risk of a reaction to heated egg, such as subjects with a history of a reaction to heated egg within the 6 months before the study were excluded. This probably explains why the median dose of heated egg eliciting a reaction during a food challenge was relatively high

TABLE IV. Anthropometric parameters in children ingesting extensively heated egg

	Baseline	3 mo	6 mo	12 mo
Height for age percentile	41.8 (11.1 to 75.2)	48.5 (17.6 to 80.9)	37.3 (12.7 to 73.1)	52.8 (17.5 to 77.9)
Height *z* score	−0.21 (−1.2 to 0.68)	−0.04 (−0.9 to 0.9)	−0.32 (−1.1 to 0.6)	0.07 (−0.9 to 0.8)
Weight for age percentile	47.7 (17.3 to 69.8)	39.3 (22.7 to 68.5)	35.6 (16.6 to 73.9)	51.1 (20.8 to 76.9)
Weight *z* score	−0.06 (−0.9 to 0.5)	−0.28 (−0.8 to 0.5)	−0.37 (−1.0 to 0.6)	−0.03 (−0.8 to 0.8)
BMI percentile	47.0 (27.4 to 71.0)	46.0 (24.8 to 73.3)	47.5 (26.3 to 73.2)	51.0 (25.5 to 75.8)
BMI *z* score	−0.08 (−0.6 to 0.6)	−0.1 (−0.7 to 0.6)	0.06 (−0.6 to 0.6)	0.03 (−0.7 to 0.7)

BMI, Body mass index.
All numbers represent median values with 25% to 75% interquartile ranges provided in parentheses. No statistically significant differences were noted in any parameter ($P > .05$).

(2.0 g), although no prior studies reported the eliciting dose for heated egg. Similarly, the relatively high eliciting dose of unheated egg (1.5 g) compared with the eliciting doses of unheated egg reported in the literature[37] is explained by "prescreening" the most allergic subjects with the challenge to heated egg and by not challenging those with test results indicating a greater than 95% probability of a reaction. We also excluded children with a known diagnosis of eosinophilic esophagitis because of the concern for the activation of the T cells with heated egg in the absence of immediate reactions.

Our final objective was to characterize the immunologic changes associated with continued ingestion of heated egg. Although we originally designed the study as a randomized trial, we were unable to recruit any subjects because of the parental concerns of no benefit to their children. Therefore subsequently all children proved tolerant to extensively heated egg were put on a diet incorporating heated egg. As a result, we do not have a comparison group for assessing the significance of the immunologic changes observed in children ingesting heated egg. Nevertheless, we are encouraged by the changes of increasing ovalbumin- and ovomucoid-specific IgG4 levels and decreasing egg white–induced SPT wheal diameters that mimic those occurring during oral immunotherapy.[38-41] Similar to children ingesting milk in baked products,[33] we observed a decrease in SPT wheal diameters and an increase in IgG4 levels within the first 3 months, with no significant further changes beyond 3 months despite continued ingestion of heated egg. The subjects in this study will continue to be followed clinically and immunologically and will undergo oral food challenges to unheated egg to determine the effect of a heated egg diet on the resolution of egg allergy.

In conclusion, the results of our study indicate that the majority of subjects with egg allergy tolerate extensively heated (baked) egg in the food matrix and that regular ingestion of those products is well tolerated and safe. Ingestion of heated egg by tolerant subjects is associated with immunologic changes that can hasten the development of tolerance to unheated egg.

Currently available laboratory diagnostic tests do not reliably predict tolerance to heated egg, and thus oral food challenges remain the only means of establishing a conclusive diagnosis. Oral challenges to heated egg must be undertaken under physician supervision with all precautions typically used for performing food challenges in children. We caution strongly against unsupervised introduction of heated egg into patients' diets. Further studies are required to determine whether ingesting heated egg affects the natural history of egg allergy when compared with strict avoidance. Until such studies are completed, there remains insufficient evidence to routinely advise the introduction of heated egg into the diet of those undertaking strict avoidance.

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Clinical implications: The majority of children with egg allergy might tolerate heated egg, but only an oral food challenge provides a definitive diagnosis. Further studies are needed to evaluate the effect of a heated egg diet on the resolution of egg allergy.

REFERENCES

1. Sicherer SH, Sampson HA. 9. Food allergy. J Allergy Clin Immunol 2006; 117(suppl):S470-5.
2. Eggesbo M, Botten G, Halvorsen R, Magnus P. The prevalence of allergy to egg: a population-based study in young children. Allergy 2001;56:403-11.
3. Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. J Pediatr 1985;107:669-75.
4. Eigenmann PA, Sicherer SH, Borkowski TA, Cohen BA, Sampson HA. Prevalence of IgE-mediated food allergy among children with atopic dermatitis. Pediatrics 1998;101:e8.
5. Wood RA. The natural history of food allergy. Pediatrics 2003;111:1631-7.
6. Savage JH, Matsui EC, Skripak JM, Wood RA. The natural history of egg allergy. J Allergy Clin Immunol 2007;120:1413-7.
7. Cooke SK, Sampson HA. Allergenic properties of ovomucoid in man. J Immunol 1997;159:2026-32.
8. Jarvinen KM, Beyer K, Vila L, Bardina L, Mishoe M, Sampson HA. Specificity of IgE antibodies to sequential epitopes of hen's egg ovomucoid as a marker for persistence of egg allergy. Allergy 2007;62:758-65.
9. Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. J Allergy Clin Immunol 1994; 93:1047-59.
10. Hoffman DR. Immunochemical identification of the allergens in egg white. J Allergy Clin Immunol 1983;71:481-6.
11. Holen E, Elsayed S. Characterization of four major allergens of hen egg-white by IEF/SDS-PAGE combined with electrophoretic transfer and IgE-immunoautoradiography. Int Arch Allergy Appl Immunol 1990;91:136-41.
12. Joo K, Kato Y. Assessment of allergenic activity of a heat-coagulated ovalbumin after in vivo digestion. Biosci Biotechnol Biochem 2006;70:591-7.
13. Deutsch HF, Morton JI. Immunochemical properties of heated ovomucoid. Arch Biochem Biophys 1956;64:19-25.
14. Urisu A, Ando H, Morita Y, Wada E, Yasaki T, Yamada K, et al. Allergenic activity of heated and ovomucoid-depleted egg white. J Allergy Clin Immunol 1997;100: 171-6.
15. Des RA, Nguyen M, Paradis L, Primeau MN, Singer S. Tolerance to cooked egg in an egg allergic population. Allergy 2006;61:900-1.
16. Eigenmann PA. Anaphylactic reactions to raw eggs after negative challenges with cooked eggs. J Allergy Clin Immunol 2000;105:587-8.
17. Knight AK, Shreffler WG, Sampson HA, Sicherer SH, Noone S, Mofidi S, et al. Skin prick test to egg white provides additional diagnostic utility to serum egg

white-specific IgE antibody concentration in children. J Allergy Clin Immunol 2006;117:842-7.
18. Boyano-Martinez T, Garcia-Ara C, Diaz-Pena JM, Martin-Esteban M. Prediction of tolerance on the basis of quantification of egg white-specific IgE antibodies in children with egg allergy. J Allergy Clin Immunol 2002;110:304-9.
19. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. J Allergy Clin Immunol 1997;100:444-51.
20. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol 2001;107:891-6.
21. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg, and peanut in children. Clin Exp Allergy 2000;30:1540-6.
22. Verstege A, Mehl A, Rolinck-Werninghaus C, Staden U, Nocon M, Beyer K, et al. The predictive value of the skin prick test weal size for the outcome of oral food challenges. Clin Exp Allergy 2005;35:1220-6.
23. A health professional's guide to food challenges. Fairfax (VA): The Food Allergy and Anaphylaxis Network; 2004.
24. Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. J Allergy Clin Immunol 1988;82:986-97.
25. Sicherer SH. Food allergy: when and how to perform oral food challenges. Pediatr Allergy Immunol 1999;10:226-34.
26. Dupont C, Barau E, Molkhou P, Raynaud F, Barbet JP, Dehennin L. Food-induced alterations of intestinal permeability in children with cow's milk-sensitive enteropathy and atopic dermatitis. J Pediatr Gastroenterol Nutr 1989;8:459-65.
27. Breslin NP, Nash C, Hilsden RJ, Hershfield NB, Price LM, Meddings JB, et al. Intestinal permeability is increased in a proportion of spouses of patients with Crohn's disease. Am J Gastroenterol 2001;96:2934-8.
28. Sampson HA. Anaphylaxis and emergency treatment. Pediatrics 2003;111:1601-8.
29. American College of Allergy, Asthma & Immunology. Food allergy: a practice parameter. Ann Allergy Asthma Immunol 2006;96(suppl):S1-68.
30. Thomas K, Herouet-Guicheney C, Ladics G, Bannon G, Cockburn A, Crevel R, et al. Evaluating the effect of food processing on the potential human allergenicity of novel proteins: international workshop report. Food Chem Toxicol 2007;45:1116-22.
31. Teuber SS. Hypothesis: the protein body effect and other aspects of food matrix effects. Ann N Y Acad Sci 2002;964:111-6.
32. Kato Y, Watanabe H, Matsuda T. Ovomucoid rendered insoluble by heating with wheat gluten but not with milk casein. Biosci Biotechnol Biochem 2000;64:198-201.
33. Nowak-Wegrzyn A, Bloom KA, Sicherer SH, Shreffler WG, Noone S, Wanich N, et al. Tolerance to extensively heated milk in children with cow's milk allergy. J Allergy Clin Immunol 2008;122:342-7.
34. Beyer K, Morrow E, Li XM, Bardina L, Bannon GA, Burks AW, et al. Effects of cooking methods on peanut allergenicity. J Allergy Clin Immunol 2001;107:1077-81.
35. Maleki SJ, Chung SY, Champagne ET, Raufman JP. The effects of roasting on the allergenic properties of peanut proteins. J Allergy Clin Immunol 2000;106:763-8.
36. Carnes J, Ferrer A, Huertas AJ, Andreu C, Larramendi CH, Fernandez-Caldas E. The use of raw or boiled crustacean extracts for the diagnosis of seafood allergic individuals. Ann Allergy Asthma Immunol 2007;98:349-54.
37. Patriarca G, Nucera E, Roncallo C, Pollastrini E, Bartolozzi F, De Pasquale T, et al. Oral desensitizing treatment in food allergy: clinical and immunological results. Aliment Pharmacol Ther 2003;17:459-65.
38. Taylor SL, Hefle SL, Bindslev-Jensen C, Atkins FM, Andre C, Bruijnzeel-Koomen C, et al. A consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? Clin Exp Allergy 2004;34:689-95.
39. Meglio P, Bartone E, Plantamura M, Arabito E, Giampietro PG. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. Allergy 2004;59:980-7.
40. Buchanan AD, Green TD, Jones SM, Scurlock AM, Christie L, Althage KA, et al. Egg oral immunotherapy in nonanaphylactic children with egg allergy. J Allergy Clin Immunol 2007;119:199-205.
41. Morisset M, Moneret-Vautrin DA, Guenard L, Cuny JM, Frentz P, Hatahet R, et al. Oral desensitization in children with milk and egg allergies obtains recovery in a significant proportion of cases. A randomized study in 60 children with cow's milk allergy and 90 children with egg allergy. Allerg Immunol (Paris) 2007;39:12-9.

TABLE E1. Baseline clinical characteristics

Patient characteristics	Heated egg reactive	Heated egg tolerant	Egg tolerant
Total no.	27 (23%)	64 (57%)	23 (20%)
Age (y), median (25th-75th interquartile range)	5.9 (5.0-9.2)	6.0 (4.1-8.0)	6.3 (4.5-10.6)
Male sex	23 (85%)	40 (63%)	14 (61%)
AD, current	17 (63%)	33 (52%)	13 (57%)
AD, resolved	7 (26%)	26 (41%)	9 (39%)
Asthma	20 (74%)	35 (55%)	18 (78%)
AR	23 (85%)	48 (75%)	19 (83%)
Family history of atopy	22 (82%)	59 (92%)	21 (91%)
Family history of egg allergy	3 (11%)	13 (20%)	2 (9%)
Breast-fed exclusively	14 (52%)	43 (67%)	17 (74%)
Time breast-fed (mo)	2 (1.5-7)*	6 (3-9)	6 (4.8-12)
Age of first reaction (mo)	13 (12-24)	12 (10-36)	12 (11.3-32.3)
Symptoms at first reaction			
Cutaneous	18 (67%)	49 (77%)	12 (52%)
UA	2 (7%)	9 (14%)	6 (29%)
LA	0 (0%)	3 (3%)	2 (13%)
GI	7 (26%)	11 (17%)	5 (22%)
History of anaphylaxis to egg	2 (7%)	9 (13%)	3 (17%)
History of EW-specific IgE >95% predictive of clinical reactivity†	22 (81%)	35 (55%)	14 (61%)
Never reacted to egg	5 (19%)	14 (22%)	8 (35%)
Additional food allergies	26 (96%)	58 (91%)	23 (100%)
Lifetime peak EW-specific IgE (kUA/L)‡§	15.4 (5.2-34.5)	6.0 (2.4-13.1)	5.9 (3.5-10.0)
Time from peak EW-specific IgE to heated egg OFC (y)‡	3.5 (2.6-4.8)	2.4 (1.0-4.3)	3.4 (2.2-7.2)
Outgrown other food allergies	13 (48%)	17 (27%)	13 (57%)

AD, Atopic dermatitis; *AR*, allergic rhinitis; *Cutaneous* (atopic dermatitis, urticaria, angioedema, pruritus, and rash); *UA*, upper airway (rhinorrhea, sneezing, coughing, and oral pruritus); *LA*, wheezing; *GI*, gastrointestinal (nausea, vomiting, diarrhea, and abdominal pain); *EW*, egg white; *OFC*, oral food challenge.

*$P = .037$, heated egg–reactive versus egg-tolerant subjects.

†Egg white–specific IgE levels of greater than 2 kUA/L (age $\leq$2 years) or greater than 7 kUA/L (age >2 years).

‡Numbers expressed as medians (25th-75th interquartile range).

§$P = .01$.